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THE ELASTIC PROPERTIES OF SILICA GELS

BY L. A. MUNRO, J. G. MCNAB, AND W. L. OTT

Abstract

The effect of age, concentration, and temperature on the Young's modulus of acid and alkaline silica gels has been measured by a compression method. For the range of concentrations used the Young's modulus is not independent of load. The modulus increases with age of the gel and with concentration. Hurd's criterion of the time of set (tilted rod method) has been evaluated in terms of absolute units. The effect of temperature on the elastic properties is different for acid and alkaline gels. The Young's modulus values for the former (pH 5.6) increase, while for the alkaline gel (pH 8.2) of the same silicate concentration the values decrease with increasing temperature. The modulus for the alkaline gel is higher than for the acid gel at the lower temperatures. A mechanism is suggested to explain these differences.

Introduction

In the setting of a gel, viscosity changes take place with time, until the sol becomes semirigid. The time of set has been defined in a number of ways, e.g., the time at which the gelling mixture will no longer flow from an inverted test tube. More consistent values are obtained by the method proposed by Hurd and Letteron (6). This measures the time for a gel to reach a rigidity that will support a glass rod 10 cm. by 3 mm. when inserted at an angle of 20° from the vertical. According to Prasad, Mehta, and Desai (13) the time of set is the time at which the extinction coefficient reaches a maximum. The latter method is of limited value with opaque, rapid-setting, alkaline gels. Other methods were used by Flemming (3) and by Batchelor (1).

It is certain that the gelation process continues beyond the time of set determined by any of the above methods. This is indicated by an increase in the rigidity with time, by syneresis, and by other phenomena.

The elastic properties of silica gel and of other inorganic gels have been recognized qualitatively for some time. However, there are in the literature very few systematic quantitative data on the elastic properties. Kröger and Fischer (9) studied the bending of rods of silica gels of one concentration of silica only (Na_2SiO_3 solution, 15% SiO_2) but containing different concentrations of acid. They reported that the elastic properties, i.e., Young's modulus, decreased continuously with a change from acid to alkaline gels. Hatschek (4) in a study of the elastic properties of several kinds of gels (agar, gelatin, and silica) determined the Young's modulus of one sample of a silica gel made by mixing 35 ml. of sodium silicate (sp. gr. 1.15) with 30 ml. of

[1] *Manuscript received June 20, 1949.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ont.

6 *N* hydrochloric acid. The pH of the gel was unstated. Using a compression method, he found that for this concentrated gel the modulus was independent of the load used and that the modulus increased with age. Hatschek concluded that, "further study of the elastic properties of gels over a large range of conditions should afford a deeper insight into their structure."

The present work was undertaken to extend the meager data at present available. The modulus of silica gels differing in concentration, pH, and age have accordingly been measured over a range of temperature by a compression method, using different loads. Hurd's criterion of "setting" has been evaluated in absolute units.

Experimental

The apparatus for the modulus determination is illustrated in Fig. 1. It consists of a vertical plunger with plates 4 cm. in diameter supported by a brass base 15 cm. in diameter equipped with three leveling screws. The movement of the plunger is kept vertical by two cross bars. This apparatus has the advantage over that used by Hatschek (4) in that the friction and the initial load due to the plunger are less. The weight of the aluminum plunger is 27.0 gm. Successive loads are applied by the addition of weights to the top plate. These weights were made by pouring slightly more than 25 gm. of molten Wood's metal into beakers and then filing the resulting disk to an accuracy of 0.01 gm.

For gels with times of set greater than five minutes, the samples were prepared by mixing suitable concentrations of sodium silicate and acetic acid solutions according to the method previously described (10). The pH was determined colorimetrically, since the glass electrode deteriorates in the presence of the silicate. The time of set was determined on separate samples by the method of Hurd and Letteron (6). For the test pieces 10 ml. aliquots were pipetted into standard vaseline-coated brass cylinders resting on a vaselined glass plate in a thermostat. For the higher temperature the glass plate was coated with a thin layer of high melting paraffin onto which the vaselined cylinder was placed immediately. Initially, the setting mixture was then covered with a thin layer of light paraffin oil to prevent evaporation. In our later experiments the oil was not added until immediately before setting. This gave a flatter surface.

For rapid-setting gels (times of set less than five minutes) 5 ml. of the sodium silicate solution was stirred vigorously into 5 ml. of acetic acid solution in the cylinders. The silicate was always added to the acid, to avoid localized gelation. Although with such a method the preparation of duplicate specimens free from inherent strains is obviously difficult, the agreement shown by the data has justified the procedure.

The above procedures gave test pieces 2.55 ± 0.05 cm. in diameter and 1.9 ± 0.15 cm. in length, with ends having almost plane surfaces. The specimens were aged in a thermostat for the desired time and then transferred to a small glass plate and placed in the instrument enclosed in a glass air

PLATE I

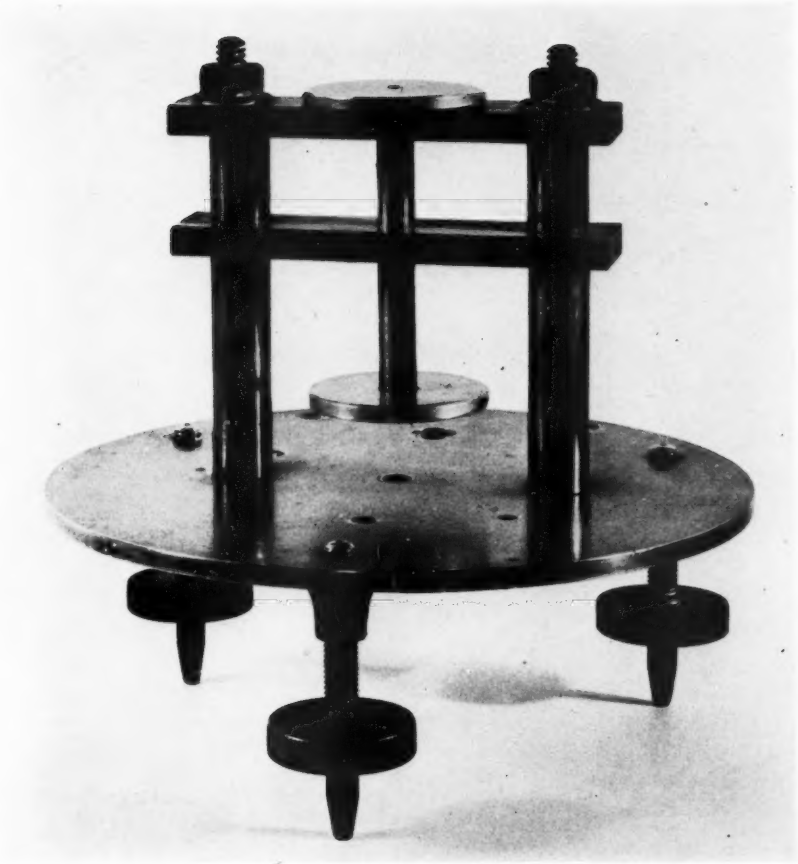


FIG. 1. *Apparatus.*

thermostat at 90 to 100% relative humidity. The dimensions of the test piece were measured by means of cathetometers. Changes in dimensions under load were measured to 0.05 mm. All readings were made 15 sec. after the load was applied.

The Young's modulus (grams per square centimeter) $= \frac{WH(1 + \Delta H)}{\pi r^2 \cdot \Delta H}$,

where W = Load in grams,

H = Height in centimeters,

ΔH = Change in height,

r = Radius of specimen.

Corrections were made for the change in cross-sectional area due to compression (5) for each load. These amount to a maximum of $\pm 6.2\%$ of the modulus values.

For duplicate gels prepared in sequence the modulus values agree within 1 or 2%. The data for gels prepared some years apart using different silicate showed a maximum deviation of 11%. Typical data for two such gels are given in Table I.

TABLE I

REPRODUCIBILITY OF YOUNG'S MODULUS (E)

pH, 5.6 Silicate conc., 4.4% SiO_2

Temp., 20° C. Time of set, 83 min.

Load, 77 gm./15 sec. readings

Gel 1—(prepared and tested 1932, J.G.M.)

Gel 2—(prepared and tested 1948, W.L.O.)

Age, hr.	Gel 1 E_1, gm./cm.²	Gel 2 E_2, gm./cm.²	Diff. $\frac{E_1 - E_2}{E_1}$, %
10	126.5	125.0	+ 1%
24	136.6	143.0	− 5%
52	153.0	153.0	0%
75	155.8	155.0	+ 0.5%
100	182.2	162.0	+11%

The Effect of Load

Hatschek reported that the modulus of the concentrated gel used in his experiments (12%) was independent of load. Unfortunately with such a high concentration a study of the relation of modulus to the time of set is impossible except at very low or very high pH values. In the present work duplicate results were obtained only when the same loads were used, as is indicated in Table II, which gives the data for a typical case. In the experiments on the effect of concentration, age, and temperature the data for a load of 77 gm. was used throughout except for the comparison of the modulus of acid gels differing in concentration. In the latter case (Figs. 2 and 3) a load of 52 gm. was used because of the fragility of the 3.3% gel. In general the determined value of Young's modulus decreases with increasing load.

TABLE II

VARIATION OF YOUNG'S MODULUS WITH DIFFERENT LOADS (E)

pH, 3.6 Silicate conc., 6.6% SiO_2
Temp., 20° C. Time of set, 364 min.

Specimen	Age, hr.	E, gm./cm.^{2} Load = 227 gm.	E, gm./cm.^{2} Load = 127 gm.
1	18	233.0	273.0
2	42	330.0	376.0
3	66	376.0	388.0
4	90	379.0	390.0
5	114	418.0	449.0
6	138	420.0	483.0

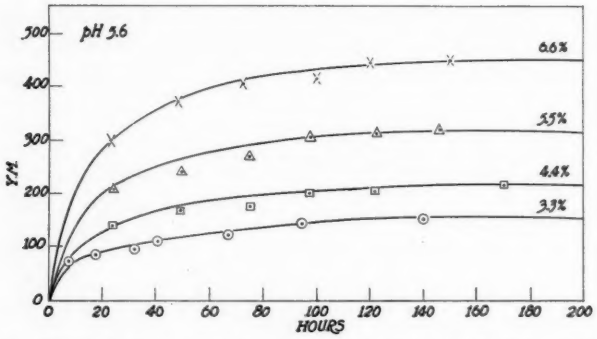


FIG. 2. *Change of Young's modulus with age for silica gels of pH 5.6 and different concentrations under 52 gm. load.*

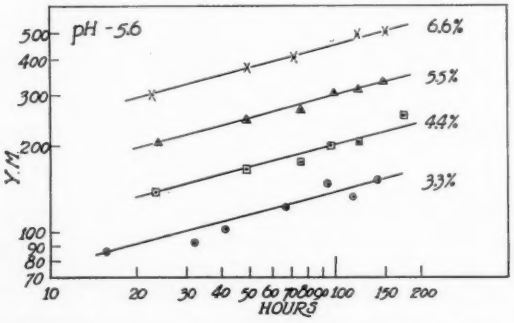


FIG. 3. *Logarithmic curves showing the change in Young's modulus with age of gels of pH 5.6 and different silica concentrations under 52 gm. load.*

The Effect of Age

Since the gelation process continues beyond the time of set, it is to be expected that the Young's modulus will increase until a constant or maximum value is reached. Fig. 2 shows the change in modulus with time, for gels of

pH 5.6, using a 52 gm. load. The numerical data for these and subsequent curves have been omitted in order to save space.

The Effect of Concentration

The dependence of modulus values on the concentration is indicated in Figs. 3 and 4 for gels of pH 5.6 and 8.2 to 8.9 respectively. In Fig. 5 the

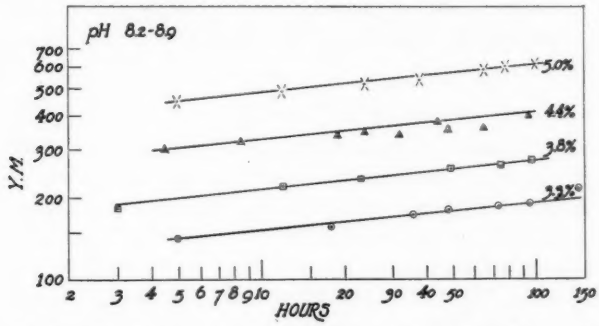
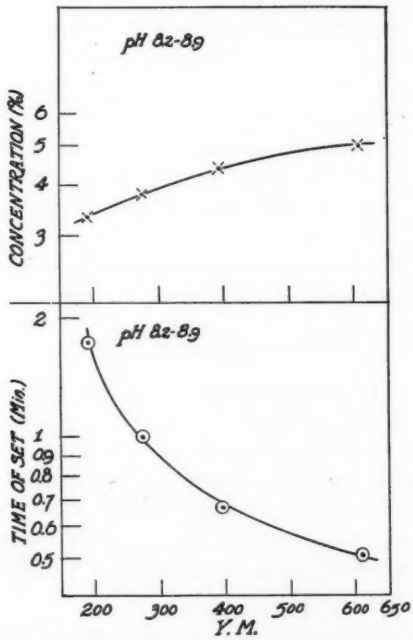


FIG. 4. *Logarithmic curves showing the change in Young's modulus with age of gels of pH 8.2 and different silica concentrations under 77 gm. load.*



FIGS. 5 AND 6. FIG. 5 (*top*). *Change of Young's modulus with silicate concentration of gels of pH 8.2 to 8.9, using 77 gm. load.* FIG. 6. (*bottom*). *The relation between the Young's modulus and time of set, using 77 gm. load.*

modulus values for 100-hr. gels are plotted against concentration and in Fig. 6 are contrasted with the times of set. The same type of curve is obtained with the 24-hr. values for the modulus. The rapid-setting gels develop the highest final modulus values.

The Effect of Temperature

The effect of temperature on the gelation process for gels of pH 5.6 and 8.2 to 8.9 is illustrated by Figs. 7 and 8. Both sets of gels were prepared from the 4.4% SiO_2 silicate.

The Relation between the Modulus and Time of Set

The tilted rod method of determining time of set used by Hurd and his coworkers is of course a measurement of the time required for the gel system to reach an arbitrary degree of rigidity, i.e., a certain modulus. Initially Hurd and Letteron (6) used a Pyrex rod 8 cm. long and 3 mm. thick. This was inserted into the gelling mixture at an angle of 20 degrees from the vertical and the time was taken when the gel had sufficient rigidity to support it. In later work (7) a 10 cm. rod was used; this would require higher modulus values as the criterion of set. Values will be constant only if the depth of gel is constant, so that beakers of the same dimensions should be used.

In an attempt to evaluate Hurd's useful criterion of the setting time of a gel in terms of absolute units, the curves for Young's modulus values versus time are extrapolated to the time corresponding to the original time of set of the gel at the given temperature. It is apparent from Figs. 7 and 8 that this procedure gives a constant modulus. For the alkaline gels (Fig. 8) the value is 156 gm. per cm.^{2} The data from which Fig. 7 was constructed were obtained several years afterwards. The values are constant but somewhat lower (122 gm. per cm.^{2}). This is undoubtedly due to the factors mentioned above. The agreement between values determined under the same experimental conditions and apparatus is surprisingly good.

Discussion

When the log time of set is plotted against the pH of the silica gel, the curve goes through a sharp minimum (11). It has been shown that the mechanism of the setting process is different for gels on opposite sides of the minimum set (12). This was indicated by the marked change in the slope of the curves log time of set vs. $1/T°A$, and by the contrast in the effect of addition agents (10). Further, syneresis, which is the exudation of dispersion medium accompanied by shrinkage of the gel, takes place rapidly with alkaline gels and slowly with acid gels of the same concentration.

In the present work one gel is on the acid side and the other is close to the pH of minimum time of set for gels of this concentration (11). In both cases the plot of the time of set vs. the reciprocal of the absolute temperature gives

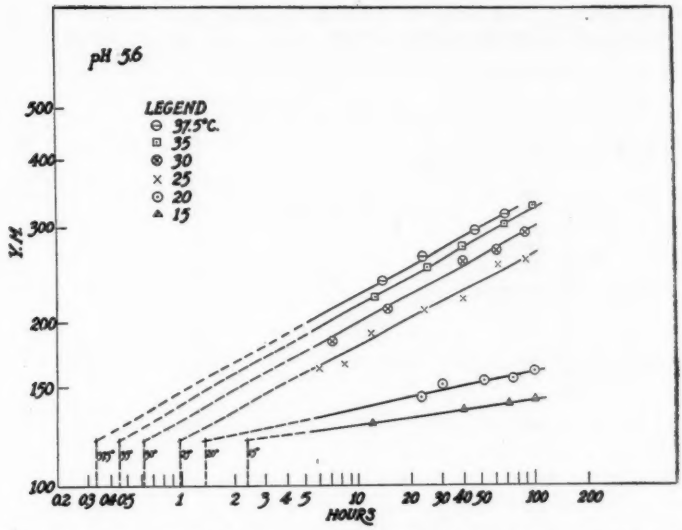


FIG. 7. *Young's modulus for gels from 4.4% SiO_2 silicate at different temperatures plotted against age and extrapolated to times corresponding to the times of set. pH 5.6. Load, 77 gm.*

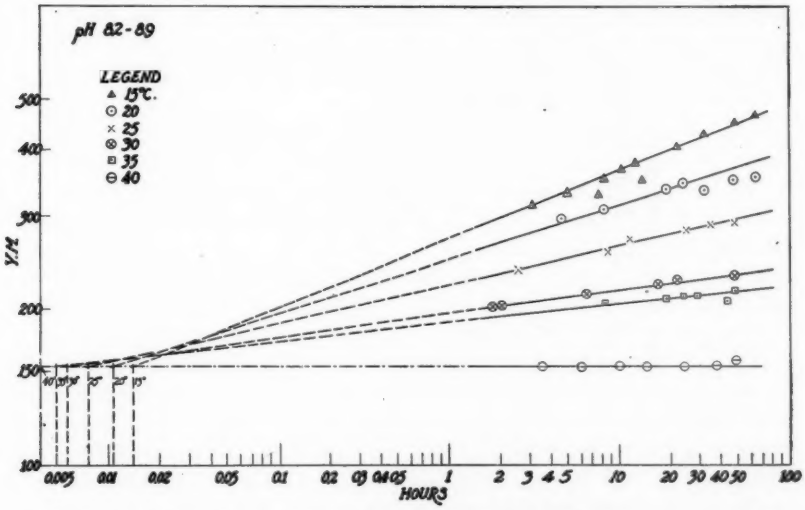


FIG. 8. *Young's modulus for gels from 4.4% SiO_2 silicate at different temperatures plotted against age and extrapolated to times corresponding to the times of set. pH 8.2 to 8.9. Load, 77 gm.*

straight lines (Figs. 9 and 10). Fig. 11 gives the modulus values at 100 hr. plotted against $1/T°A$. The curve for the acid gel shows an increase with increasing temperature while the curve for the alkaline gel shows a decrease.

An unexpected observation is that the Young's modulus of the alkaline gel at room temperature is higher than that of the acid gel of the same silicate concentration. The recovery of the alkaline gel on removal of the applied

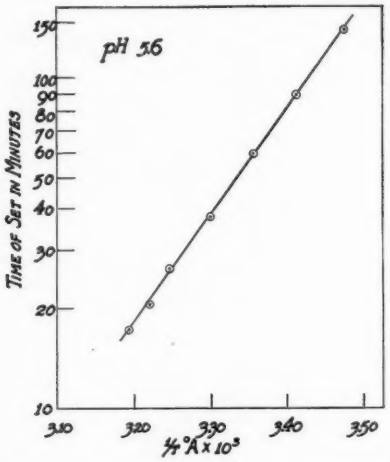


FIG. 9. *Variation of time of set with the reciprocal of the absolute temperature for silica gels of pH 5.6 from 4.4% SiO_2 silicate.*

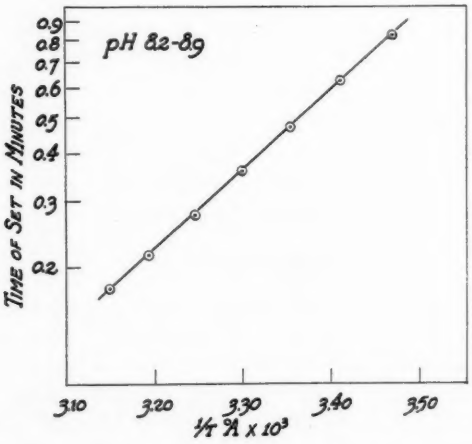


FIG. 10. *Variation of time of set with reciprocal of the absolute temperature for silica gels of pH 8.2 from 4.4% SiO_2 silicate.*

load is less than that for the acid gel, i.e., the elasticity is less. The above facts and the difference in the effect of temperature on the modulus of acid and alkaline gels must be explained by any suggested mechanism.

It has been shown (8, 12) that for gels of pH greater than 8.5 (approximately) the plot of log time of set vs. $1/T°A$ deviates from a straight line; this indicates a second effect in the gelation process.

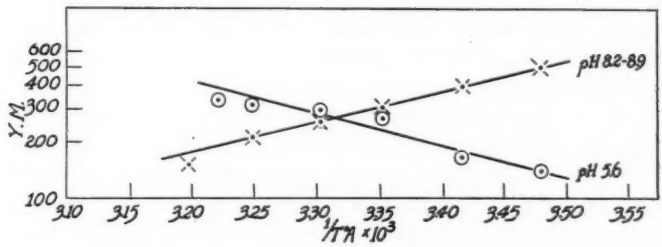


FIG. 11. *The 100 hr. Young's modulus values for an acid and an alkaline gel plotted against the reciprocal of the absolute temperatures. Silicate, 4.4% SiO_2. Load, 77 gm.*

Carman (2) has suggested that in alkali media there is a strong tendency toward the destruction of the Si–O–Si links, with the production of smaller aggregates. Hurd (8) considers that "strong alkali reverses the process which occurs during gelation". He believes that "alkaline gels are softer and less elastic than acid gels since condensation and cross linkage formation probably do not proceed so far in alkaline gels."

While the straight line in Fig. 10 would suggest that the degradation of the polymer or peptizing effect of the alkali is not as pronounced as at higher pH values, nevertheless Fig. 11, which shows that the modulus of the 8.2 gel decreases with increasing temperature, would indicate that this effect is present.

The increase in modulus with temperature for the acid gels (Fig. 11) can be explained by the production of additional linkages by further condensations at the higher temperatures. This is in agreement with the observation of Schwarz and Stöwener (14) that acid silica gels prepared at 100° C. adsorbed less methylene blue than a gel prepared at 0° C., which observation is interpreted as meaning that the total surface of the high temperature gel is less than that of the low temperature gel. If the increase in modulus were due to a larger number of smaller particles, as is obtained in linear polymerization at higher temperatures, the adsorption would be *greater*, not less.

It is well known that freshly precipitated silica gel may sometimes be peptized by acid or alkali while an aged gel cannot. This also indicates that the observed increase in Young's modulus with age is not associated with an increase in total surface such as would be obtained if additional small polymers were formed. It rather points to cross linkages, as suggested above. Indeed, it has long been observed that in many instances sols increase in particle size by forming chainlike or branched structures. The modulus of all gels increased with age.

The fact that the modulus of the alkaline gel at the lower temperature is *higher* than that of the acid gels of the same concentration may indicate that in

the alkaline medium gelation produces the Von Weimarn ($BaSO_4$) type of gel with a higher number and less-linear polymers, i.e., three dimensional polymers of small particle size, and these give, like bentonite, a stable structure different from that of the acid gels. This is somewhat analogous to the known effect of acid and alkali on the condensation of phenolic resins although the processes are dissimilar. At higher temperatures the peptizing effect of the alkali becomes greater and reduces the Young's modulus.

The above mechanism is in accord with the more elastic recovery of the acid gels when the load is removed and with the more rapid syneresis and shrinkage of alkaline gels as well as the marked differences in optical properties.

Houwink (5) cites the observations of Kowalski that the modulus of glass decreased with the temperature whereas Sosman (15) reported an increase. The latter differences are not analogous to those indicated in the present work, in that the experiments on glass were carried out on specimens formed at the same temperatures whereas our results were obtained with samples kept at the recorded temperature throughout the whole gelation period.

It is to be expected that if the medium is made more alkaline, the predominance of cross linkages would no longer compensate for reduced polymer size and the modulus would accordingly decrease. Measurements on more alkaline gels are therefore suggested. Some anomalous results were obtained with the acid gels at higher temperatures. It might be expected that at a certain temperature thermal vibration might cause breaking of the loosely linked structures in the acid gels and cause a decrease in the modulus values. The extension of the study to higher temperatures is desirable.

Acknowledgment

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LIGHT-SCATTERING APPARATUS AND TECHNIQUES FOR MEASURING MOLECULAR WEIGHTS OF HIGH POLYMERS[1]

By H. J. Hadow, H. Sheffer, and J. C. Hyde

Abstract

Apparatus is described for the measurement of small amounts of scattered light, dissymmetry of radiation, depolarization, and very small refractive index differences. Methods of calibration are critically examined. Benzene was adopted as a turbidity standard. Measurements were made on fractionated polystyrene of known molecular weight and on bovine serum albumin solutions. Values of the reduced intensity for benzene were obtained:

$R = 33.4 \times 10^{-6}$cm.$^{-1}$ at 25° C. and $\lambda = 4358$ Å, and

$R = 12.4 \times 10^{-6}$cm.$^{-1}$ at 25° C. and $\lambda = 5461$ Å.

Degree and sources of error are discussed

Debye (7, 8) has developed the theory of light-scattering in solutions and has produced an equation that relates the molecular weight of the solute to experimentally obtained values of turbidity, refractive index increment, and concentration. This equation, in its most popular form, is

$$H\frac{c}{\tau} = \frac{1}{M_2} + 2Bc\,, \qquad (1)$$

where c is the concentration in grams per milliliter, τ is the turbidity or extinction coefficient in cm.$^{-1}$, M_2 is the weight-average (16) molecular weight of the solute, B is the constant that is used as a measure of deviation from ideality in the van't Hoff osmotic pressure equation and

$$H = \frac{32\pi^3}{3}\frac{n_0^2}{N\lambda^4}\left(\frac{dn}{dc}\right)^2,$$

where n_0 is the refractive index of the solvent, N is Avogadro's number, λ is the wave length of the incident light and $\frac{dn}{dc} = \frac{n - n_0}{c}$ for dilute solutions, n being the refractive index of the solution.

Several methods for measuring τ and $\frac{dn}{dc}$ have been described in the literature (e.g., 3, 9, 12, 26, 27, 28). This article deals with a few innovations and improvements that have been made in the apparatus and techniques. The main objective, however, will be to point out the difficulties that have been encountered, in the hope that others who wish to construct this type of equipment may avoid many time-consuming incidental investigations.

The Measurement of τ

For fairly turbid solutions, the extinction coefficient may be determined directly by transmission measurements, using Lambert's law. The use of the Beckman spectrophotometer for such measurements is discussed below under

[1] *Manuscript received May 2, 1949.*

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"Calibration". The turbidity of dilute solutions of most polymers is too low to be measured in this way since the difference in transmission between solvent and solution is very small. The easiest way to determine these turbidities is to measure the light scattered at right angles to the incident beam by the solution and by the solvent. The turbidity determined in this manner must be corrected for the effect of intramolecular interference when the length of the solute particles exceeds $\frac{\lambda}{20}$ (16, 21, 28).

The measured turbidity contains a contribution due to fluctuations in orientation of the molecules. This may be calculated if a measurement of the depolarization is made. The depolarization $\rho = \frac{H}{V}$, where H is the horizontal component and V the vertical component of the scattered light when the incident light is unpolarized. The depolarization has been neglected in Equation (1), but for accurate work and especially for large anisotropic molecules the molecular weights obtained from this equation must be corrected by multiplying by the inverse of the Cabannes factor, $\frac{6 + 6\rho}{6 - 7\rho}$. In most cases this reduces the value obtained for the molecular weight by less than 10%. For certain solvents (e.g., benzene and toluene) the depolarization must be corrected for the contribution of the solvent (11).

When the dimensions of the solute particles exceed $\frac{1}{20}$ of the wave length of the incident light, interference between the light waves scattered from different parts of the macromolecule brings about a change in the shape of the radiation envelope. More light is then scattered in the forward than in the backward direction (16). This causes the measured turbidity to be lower than the true value. The correction factor may be calculated if the dissymmetry (i.e., the ratio of the forward to backward intensities at equal angles about 90°) is measured. The theoretical variation of intensity with angle has been worked out for solute particles in the shapes of rods, spheres, or random coils (9, 16, 28). If the polymer under consideration is known to fit one of these models, the correction factor can be accurately obtained from the dissymmetry data and may increase the measured turbidity by over 100% in some cases (21). The length of the molecule can also be calculated from dissymmetry data (10, 16, 21). A satisfactory apparatus for obtaining molecular weights of large polymer molecules by light-scattering must, then, provide facilities for the measurement of (1) intensity of light scattered at right angles to the incident beam, (2) dissymmetry of the scattered radiation, and (3) depolarization of the light by the solution.

Light-Scattering Apparatus A

(*i*) *Optical System*

A schematic diagram of the apparatus is shown in Fig. 1. The light source is a General Electric AH-4 mercury lamp cooled by a small fan. By

means of a lens an image of the source is focused on a diaphragm with an opening of about 2 mm. diameter. A second lens is placed at a distance equal to its focal length from the diaphragm to give a beam of substantially parallel light.

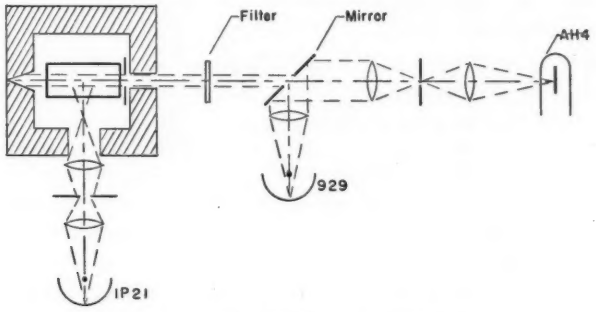

FIG. 1. *Light-scattering apparatus A.*

The light-beam then passes through color filters. The 5461 Å line was isolated as closely as possible by one set of filters, the 4358 Å line by another. The response of the system to different wave lengths is affected by (*a*) the color filters used, (*b*) the intrinsic response of the photosensitive surface, and (*c*) the relative intensity of the different wave-length bands in the light from the source. For the 5461 Å line a Corning filter No. 3484 is used to eliminate all wave lengths below 5200 Å, with a No. 5120 filter to eliminate the strong band at 5770 Å in the mercury spectrum. This filter combination would pass light of wave lengths greater than 6000 Å, but the S-4 surface of the photomultiplier that was used to measure scattered intensity is decreasingly sensitive to increasing wave length in this region. The source produces a continuous spectrum of intensity about 2 to 3% of that of the 5461 line. It is estimated that the system is influenced by the continuous spectrum over the range 5200 to 5700 Å, which is passed by the filters, but the response is less than 3% of that caused by the 5461 Å line. The effect of other wave lengths is negligible. For the 4358 Å line, Corning filters No. 5113 and No. 3389 are used.

It was desired to reflect a portion of the incident beam onto a 929 phototube linked to the photomultiplier circuit in such a manner as to compensate for fluctuations in the output of the lamp. The circuit is described in detail below. At first the simple procedure, adopted by Doty, Wagner, and Singer (12), of placing a pair of parallel glass plates at 45° to the beam was used. It was found, however, that this partially polarized the beam, necessitating, as a compensator, the insertion of a "depolarizer" of two further plates at 45°, but reflecting on an axis at 90° from the first pair. Since these plates decreased the intensity of the beam considerably, the method of Zimm (29) has recently been adopted. This involves simply the use of a silvered glass plate with a hole in the center. Most of the beam passes through the hole, and the small fraction striking the plate is reflected onto the 929.

The main portion of the beam then passes through a hole 1 cm. square into the cell chamber, which is a thermostatically controlled box of inside dimensions 4 by 4 by 6 in., the outside dimensions being $1\frac{1}{2}$ in. greater all around. Water from a thermostat can be pumped through the walls, bottom, and lid of the box. After passing through the solution cell the beam is finally trapped in a blackened cone.

The scattered light is picked up by a lens and focused on a slit slightly smaller than the photosensitive surface, an image of the slit being focused by an additional lens onto the photomultiplier. The light must not be focused directly to a small spot on the sensitive surface, as this causes rapid local fatiguing and nonlinearity of response. In addition, any slight movement of the parts would move the spot on the sensitive surface; this is to be avoided, as the sensitivity varies over the surface. The latter point has been emphasized recently by Zimm (29).

When using solutions scattering large amounts of light, the photomultiplier is protected by the use of a 1.3 neutral density filter in the incident beam. By calibration, both on the apparatus and on a spectrophotometer, the filter reduced the intensity by a factor of approximately 1 : 24 for $\lambda = 5461$ Å, and 1 : 38 for $\lambda = 4358$ Å.

For the measurement of depolarization, polaroid disks can be inserted in the paths of the incident and scattered light beams. With suitable photomultipliers (see below) much more accurate determinations of depolarization at low light intensities can be made than by visual methods such as the Cornu method (11).

(ii) Cells and Dissymmetry Device

Rectangular glass cells of dimensions 4 by 2 by 8 cm. were used. For measurements of 90° scattering alone, these cells could be used with the 4 cm. sides parallel to the incident beam. For use with the dissymmetry device, however, it was necessary to cement a glass slide on the 4 by 2 cm. opening, drill an opening in one of the 8 by 2 cm. sides and use the cell with the 8 cm. sides parallel to the incident beam. There was no difficulty in obtaining suitable cements for aqueous solutions, but for organic solvents such as benzene the many cements tried proved very unsatisfactory except for a gum arabic – sucrose – water cement suggested by Dr. Marcel Rinfret (24).

For the measurement of dissymmetry, a brass block carrying two small front surface mirrors (Fig. 2) was used. The block fitted snugly on two pins in a position parallel to the cell and about 1 cm. in front of it. In one position the angle of view in air was 43° from the direction of the incident beam; with the block turned end over end the angle was 137°. Allowing for refraction at the cell wall, the angles with benzene in the cell were 63° and 117°. A diaphragm was placed so that only light from the center portion of the second mirror was viewed by the photomultiplier.

The cell holder, comprising a rectangular recess for the vessel with inlet and exit diaphragms, was of sheet brass soldered to a brass plate. This assembly, together with the dissymmetry block, could be removed from the thermo-

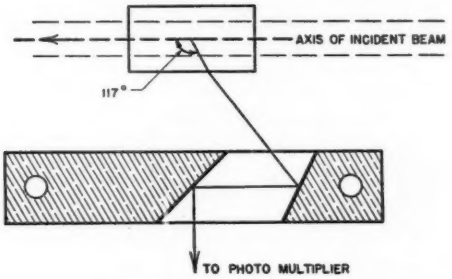

FIG. 2. *Dissymmetry device.*

statically controlled box. All interior surfaces were painted flat black. Additional shielding around the cell holder was found to be necessary to eliminate stray light, which was particularly likely to interfere with the dissymmetry measurements. With clean benzene in the cell, dissymmetries varying by 1 to 2% from 1.0 were obtained. Small variations were due to imperfections in the cell walls and the degree of cleanliness of the surface.

(iii) The Photoelectric System

Since it is desirable to use a photomultiplier with an S-4 surface for greatest sensitivity in the blue-green region, one has to choose between the 931-A and 1P21 photomultiplier tubes. The latter is much more expensive, but has definite advantages, particularly in so far as stability and low dark current are concerned. Comparison between three 1P21's and five 931-A's is given in Table I. The last column gives the measured depolarization of benzene

TABLE I

CHARACTERISTICS OF PHOTOMULTIPLIERS

Tube No.	Sensitivity relative to 1-P21-A	Dark current, $\times 10^9$ amp.	ρ for C_6H_6
1P21-A	1.00	3	0.42
1P21-B	0.32	3	0.44
1P21-C	0.42	3	0.42
931-A-1	0.14	40	0.37
931-A-2	1.09	70	0.38
931-A-3	0.37	20	0.38
931-A-4	0.07	10	0.34
931-A-5	0.17	90	0.40

for each tube. It may be seen from this table that the sensitivities of the best 1P21 and the best 931-A are about the same, but in every case the dark currents of the 1P21's are much lower than those of 931-A's. In this regard it is necessary to illuminate the new tubes strongly for some time until the dark current is reduced to a low steady value. Even with this precaution there are always some fluctuations in the dark currents of the 931-A's.

A value of 0.420 is given by Cabannes (4) for the depolarization of benzene. It appears that the 1P21's duplicate this value fairly closely, while the 931-A's give varying low values indicating that they are more sensitive to vertically than horizontally polarized light. This is particularly difficult to understand, since, according to Johnson (14), 1P21's are the selected 10% of the 931-A's having low noise and stable sensitivity.

All phototubes fatigue or recover during use, depending on the intensity of light being measured. This necessitates repeated reference to turbidity standards in light-scattering work. Since the drift increases with output, it was found a desirable precaution to keep the output below 5μa., which is considerably lower than the figure suggested by Engstrom (13).

For the satisfactory use of a photomultiplier in the light-scattering apparatus it is necessary to have a supply of regulated high voltage to be applied to the stages of the multiplier and also to eliminate or compensate for fluctuations in intensity of the light source. The basic circuits to meet these requirements were designed by Dr. R. B. Harvey of these laboratories and are shown in Fig. 3.

The high tension voltage regulation embodies the principles of Bereskin (1). V_4 consists of five type 991 voltage regulating tubes which draw about 1 ma. The bridge $R_6R_7R_8$ draws approximately $\frac{1}{2}$ ma. The approximately 1000 v. are divided up between the stages of the multiplier, slightly over 100 v. being applied to each stage except the last one. To increase the stability only 50 v. are applied to the last stage.

Fluctuations in the intensity of the light source cannot be eliminated entirely by control of the voltage applied to the lamp. Variation in the temperature of the lamp, local hot spots, and aging are additional sources of fluctuations. The 929 photocell was used to compensate for these by adjusting the amplification of the photomultiplier (19) in the manner shown in Fig. 3. The 929, which receives a small amount of light from the incident beam, was connected between dynode 4 and dynode 2 of the photomultiplier. Dynode 4 was connected to the arms of R_{19} through a 5 meg. variable resistor R_{20}. Now, if the voltage of one dynode is plotted against the output of the photomultiplier a peaked curve is obtained owing to defocusing of the electron beam at voltages greater or lower than the optimum. If the voltage of the dynode in question is set at such a point that an increase of voltage causes the over-all amplification of the photomultiplier to increase while at the same time the increase of voltage

on the dynode causes defocusing and a decrease of the output, then the net output will tend to remain stationary. In this case it was found experimentally that varying the voltage on dynode 4 gave the most satisfactory results. R_{19} allows the voltage on dynode 4 to be put on the descending portion of the output-voltage defocusing curve. The flow of current in V_2 causes the dynode end of R_{20} to become negative. Now an increase in the intensity of the light

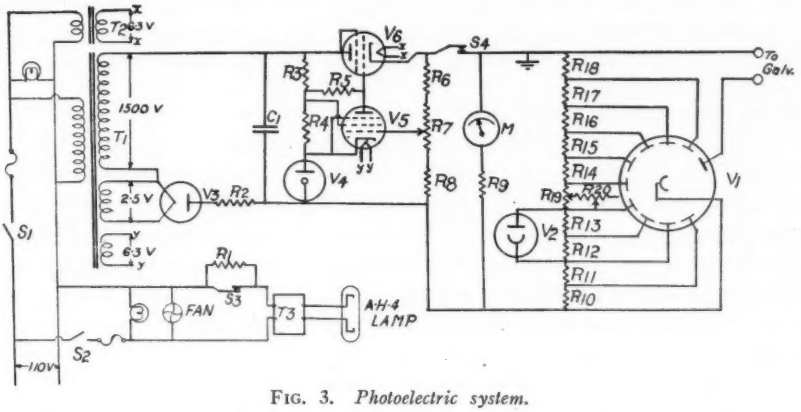

FIG. 3. *Photoelectric system.*

Parts list

V1	*1P21 Photomultiplier*	*R1*	*3 ohms, 7.5 w.*
V2	*929 Phototube*	*R2*	*50k ohms, 10 w.*
V3	*2X2 Rectifier*	*R3*	*1 megohm, 2 w.*
V4	*5-991 Voltage regulators*	*R4*	*250 k ohms, 1 w.*
V5	*6SJ7*	*R5*	*2 meg.*
V6	*6V6*	*R6*	*3 meg.*
T1	*Hammond 215 C.R.O. transformer*	*R7*	*100 k ohm pot.*
T2	*Hammond 167C transformer*	*R8*	*1 meg.*
T3	*G.E. Autotransformer for AH4 lamp*	*R9*	*2 megohms*
S1	*H.T. switch*	*R10–R17*	*100 k ohms*
S2	*Mercury lamp switch*	*R18*	*50 k ohms*
S3	*Push button—normally closed*	*R19*	*100 k pot.*
S4	*Microswitch—normally open*	*R20*	*5 meg. pot.*
M	*Meter 0–1 ma.*	*C1*	*1 μf. 2000 W.V.*

source causes the output of V_1 as a whole to increase. Simultaneously it causes the current through V_2 and R_{20} to increase; this makes the dynode end of R_2 more negative and forces the amplification down. When the effects are balanced, fluctuations of the light source have a minimum effect on the output. The circuit was adjusted so that a 10 v. change in the light source supply had a negligible effect on the output reading. When S_3 is opened R_1 causes a drop of about 6 to 8 v. in the supply voltage and aids in setting R_{19} and R_{20} to compensate for fluctuations in light source intensity. These settings rarely have to be changed during operation.

The microswitch S_4 breaks the circuit automatically when the lid of the cell chamber is opened. This eliminates possible overloading of the photomultiplier and galvanometer by admitting external light with the high tension still applied.

The output of the photomultiplier is measured directly on a Rubicon galvanometer with a 100 mm. scale and a sensitivity of 0.005 μa. per mm. An Ayrton shunt is used when necessary with the larger currents. The sensitivity of the instrument in terms of turbidity is about 1×10^{-6}cm.$^{-1}$ per mm. galvanometer deflection when using photomultiplier 1P21-A.

Light Scattering Apparatus B

For measuring the dissymmetry of large molecules it is desirable to use angles closer to the incident beam than was possible with Apparatus A (20). It is also desirable to measure the dissymmetry at more than one angle, and at more than one wave length, to determine whether a satisfactory model has been chosen for the molecule. For these reasons a second apparatus (*B*) was designed as shown schematically in Fig. 4.

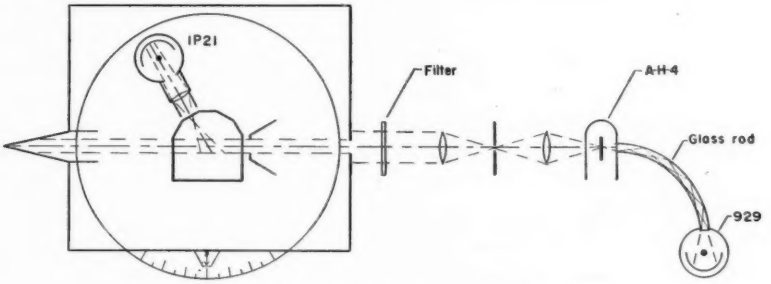

FIG. 4. *Light-scattering apparatus B.*

The cell, made by Klett, was of optical glass fused at the corners. The viewing angles were 33, 60, 90, 120, and 147°. Each of the viewing faces was 12 mm. wide, the entering and exiting faces were 30 mm. wide, and the back face close to 50 mm.; the height was 100 mm. The cell was mounted on a platform about which the photomultiplier could be rotated so that the scattered light at each angle was viewed at 90° to the face. Thus no correction was required for the refractive index of the liquid. Direct transmission measurements could also be made. The photomultiplier was mounted on a rotating arm attached to a divided circular scale. Part of the scale protruded from a false bottom in the blackened box containing the cell and multiplier.

The optical system was similar to that in Apparatus *A*. A 6 mm. square slit about 4 cm. from the cell defined the light beam and it was found necessary to insert a slightly larger slit close to the cell in order to remove any divergent portion of the beam.

This apparatus could be connected into the electrical circuit of Apparatus *A* by means of a 12-pin Jones plug. In this case the AH-4 lamp was enclosed in a water-cooled jacket from which a portion of the light was led off to a 929 phototube by means of a bent glass rod.

Calibration and Measurements

Attempts were made at first to calibrate the apparatus by means of a magnesium carbonate surface at 45° to the light beam as described by Stein and Doty (28). The size of the opening through which the photomultiplier views either the illuminated area of the block, which is of a size fixed by the width of the beam, or the luminous zone in the solution, which is limited only by the length of the cell, is obviously extremely critical. In addition there is a certain amount of specular as well as diffuse reflection from the block at this angle.

This method was then discarded in favor of that used by Doty, Affens, and Zimm (10) in which the turbidity standard was a solution of polystyrene in ethyl methyl ketone which was calibrated on a Beckman spectrophotometer. It is necessary to use solutions that exhibit no dissymmetry; this means that the molecular weight of the polystyrene used should not be much greater than 50,000. Great care must be used in obtaining the matching factors of the cells (25) and in the handling of the solutions. Since the turbidities are fairly low it is very easy to introduce errors of the order of 10% in turbidity by errors of 0.2% in transmission on the Beckman. In addition, it was found most difficult to remove the last "motes" from the fairly large volumes of solution required, either by centrifuging or by filtering through fine or even ultrafine filters. Any handling, particularly pouring from one vessel to another, introduced many dust particles. It is necessary to make a correction for absorption of the light over the path traversed in the cell of the light-scattering apparatus. Since the standard has a much higher turbidity than the solutions to be measured, this correction may be of the order of .5%. Another difficulty was a change of the ethyl methyl ketone with time. It is a desirable solvent to use in these measurements because the $\frac{dn}{dc}$ for polystyrene in ethyl methyl ketone is very high. However, the solvent polymerizes and readily develops a greenish cast that is difficult to remove. In any case, such solutions calibrated on the Beckman were found to have turbidities 10 to 30% too high when compared with benzene as a standard of turbidity on the light-scattering apparatus.

It would appear, therefore, that a solvent of relatively high scattering power such as benzene or carbon disulphide would serve as the best standard of turbidity. The turbidities of these solvents have been measured with a reported accuracy of about $\pm 6\%$ (4, 5, 6, 22). Such values can also be obtained using the light-scattering apparatus if a material of known molecular weight is used as a reference standard. Carbon disulphide has the higher turbidity and was found satisfactory for use with green light, but not with

blue, since it is apparently sensitive to light of lower wave length (4). Benzene was chosen as the standard, since much of the work planned for the apparatus had to do with benzene solutions. Reagent grade benzene was redistilled twice through a packed column, the middle fraction being kept each time. The product was found to be quite free from motes when observed at angles close to a beam of light passing through it. A suitable quantity of the solvent was sealed in appropriate cells for use in both light-scattering apparatus *A* and *B*.

When using a solvent in this manner as an absolute standard of turbidity, it is necessary to learn if the cell itself contributes appreciably to the light scattered. This cannot be determined from measurements on the empty cell because internal reflections then make the value unrepresentative. An estimate of the contribution may be obtained by viewing the cell filled with "clean" water which has a very low scattering power (4). Comparing the scattering power of benzene with that of water in the present equipment it was estimated that any contribution of the cell was below 2% of that of benzene. Imperfections in the cell walls, however, caused variations of the order of 2% in the readings for benzene in different cells. In this regard it was found most important to highly polish the cell walls, as any microscopic contamination picks up dust particles which reflect light to the multiplier. It should be emphasized that the size of the cells, the position of the beam through the cells, and the viewing angles were chosen to avoid reflections from corners and interference from the spot that is brightly illuminated by the incident beam.

Having adopted benzene as a turbidity standard, the technique of measuring the turbidity of unknowns must now be considered. In this connection the most important point is the "cleaning" of the solutions since it is obvious that dust particles increase the turbidity and especially the dissymmetry. Since large cells were deliberately chosen for the reasons mentioned above, a minimum of 40 cc. of solution was required in each cell. It has also been previously noted that cleaning such amounts of solution by filtering was found quite unsatisfactory. Not only is it difficult to remove fine motes by this method, but in addition an analysis of the solution is required after filtering. For example, in one extreme case a Berkfeld candle filter removed over 75% of the polymer present in solution. Even small sintered glass filters removed as much as 10%. For the solvents themselves ordinary distillation proved very satisfactory in the case of organic solvents. Water, however, presents a much more difficult problem and it was found that freshly distilled water from a good still looked very "dirty" when examined in a Tyndall beam.

Of all the methods tried, only the one described by Bhagavantam (2) was found at all successful in removing the "motes" from water. Water was distilled from a warm bulb to a cold one under vacuum, and after rinsing the cold bulb returned again for repeated distillations. Air was slowly allowed into the warm bulb, the tube connecting the bulbs broken and the purified water poured into the light-scattering cell. The method was found to be quite tedious and not always completely successful.

The following operating procedure was finally adopted as being the most satisfactory of those investigated. A Sorvall SS-1 angle centrifuge (12,000 r.p.m.) was used to clean the solutions. The ordinary round-bottomed tubes provided with the instrument are not very satisfactory for this work because the sediment is easily stirred up by convection currents when the centrifuge is stopped and particularly by any jarring on removal of the tubes. To diminish this effect the 15 cc. heavy-walled Pyrex tubes were drawn down and sealed to a 1.5 mm. capillary tube about 3 cm. long. The resulting tube, which was very similar in appearance to a Hopkins Vaccine Tube, had a capacity of about 6 cc. Polyvinyl alcohol caps were made for use with organic liquids. For turbidity measurements, the eight tubes were usually filled with a 1% solution and, after centrifuging, 1 cc. portions were removed by pipette from each tube in turn to be added to the 40 to 50 cc. of distilled solvent in the light-scattering cell. With this procedure eight points in the concentration range 0.02 to 0.2 gm. per 100 cc. could be obtained. The centrifuge was kept running between each measurement. Readings for benzene in the standard cell were taken before and after each solution measurement. To avoid evaporation, glass slides in which an 8 mm. hole had been drilled were cemented to the cells and the hole plugged with a suitable cap. Although the volumes of solvent used were large, the actual amount of polymer necessary for the measurements was quite small. To facilitate calculations it was found advisable to set the galvanometer reading for benzene at a predetermined value by adjusting the potentiometer R_7 (Fig. 3). This galvanometer reading then corresponded to the turbidity value for benzene and the reading for the unknown yielded the uncorrected turbidity directly after subtraction of the contribution of the solvent. Dissymmetry and depolarization corrections were obtained in the manner previously described.

The first check on the apparatus was to measure the ratio between the scattered intensities for carbon disulphide and benzene. Ratios of 4.3 to 4.4 were obtained which may be compared with the values of 4.38 obtained by Martin (17), 4.54 by Martin and Lehman (18) and 4.12 by Krishnan (15).

It was necessary to check the validity of using benzene as a standard by the use of material of known molecular weight. For this purpose a sample of polystyrene was prepared by polymerization at 175° C. Fractionation was carried out by the standard method of precipitation from very dilute solutions. Each fraction was reprecipitated and one of the fractions was carefully refractionated for this work. The intrinsic viscosity of the final fraction differed from that of the previous one by only 0.5%; this indicated that the molecular weight distribution must be very narrow. From the very accurate osmotic pressure measurements of Dr. L. A. McLeod of the National Research Laboratories, the molecular weight of the final narrow middle fraction was determined to be 141,000. Benzene solutions of this fraction were then examined at 25° C. on both light-scattering apparatus *A*

and B at $\lambda = 4358$ Å and 5461 Å. Results from a number of experiments were averaged; this gave $\left(\frac{c}{\tau_{rel}}\right)_{c=0} = 1.02 \times 10^{-3}$ for the blue line and 0.946×10^{-3} for the green line. The values for τ_{rel} must be multiplied by the corresponding turbidity of benzene for conversion to absolute values and also corrected for dissymmetry.

Values of the turbidity of benzene at 25° C. for the 5461 Å line do not appear to be available in the literature. However for the 4358 Å line at 24° Peyrot (23) gave a value of $(34.8 \pm 2.3) \times 10^{-6}$ for the reduced intensity R, corresponding to $\tau = 5.83 \times 10^{-4}$cm.$^{-1}$ $\left(\tau = \frac{16}{3}\pi R\right)$. The variation of the reduced intensity of benzene with temperature is given by Cabannes as an increase of 0.7% per degree, so that the temperature correction may be neglected here. This number for the turbidity of benzene may then be used to change the τ_{rel}'s obtained for the polystyrene solutions into absolute turbidities. To obtain the dissymmetry correction the measured dissymmetries of the polystyrene solutions were plotted against concentration and a value of 1.08 was obtained by extrapolation to infinite dilution. The measured turbidity must then be increased by 4%, assuming a random coil model for the polystyrene (10, 28). Inserting the corrected value for $\left(\frac{c}{\tau}\right)_{c=0} = 1.685$ in Equation (1) along with the determined value of $\frac{dn}{dc} = 0.106$ (see below), a molecular weight of 155,000 was obtained. This must now be reduced by 5% because of the depolarization correction, the measured depolarization for very dilute solutions being $\rho = 0.027$. A final value of 147,000 (as compared with 141,000 by osmotic pressure) was obtained. Excluding experimental error, these values should be the same if the assumption is made that the molecular weight distribution of the polystyrene fraction is so narrow that the number-average and weight-average molecular weights are nearly identical.

It is probable that the value obtained by osmotic pressure measurements for the molecular weight of the polystyrene fraction is more reliable than the number for the reduced intensity of benzene used in the above calculations. It is therefore desirable to recalculate the latter from the present data. Assuming 141,000 to be the correct molecular weight, R for benzene at 25° C. and $\lambda = 4358$ Å should equal 33.4×10^{-6}cm.$^{-1}$. Cabannes (5) has calculated a value of R at 15° C. for this wave length; his figure when corrected to 25° C. is approximately 29.3×10^{-6}. This is considerably lower than Peyrot's value (34.8×10^{-6}) and the one determined in this manner from the light-scattering data. Working backwards from the measured $\left(\frac{c}{\tau_{rel}}\right)_{c=0}$ for $\lambda = 5461$ Å and assuming that 141,000 is the correct molecular weight, a value for the reduced intensity of benzene at 25° C. and for this wave length was obtained, $R = 12.4 \times 10^{-6}$cm.$^{-1}$. Zimm (29) has estimated a value

of 13×10^{-6} for R at 20° C. and $\lambda = 5460$ by using the data of Peyrot and the value of $R = (10.7 \pm 0.55) \times 10^{-6}$ for $\lambda = 5440$ Å and a temperature of 15° C. determined by Cabannes and Daure (6). This is equivalent to $R = 13.4 \times 10^{-6}$ at 25° C. and $\lambda = 5460$. This estimate is considerably higher than our calculation of $R = 11.2 \times 10^{-6}$ from the same data. The value determined experimentally from the light-scattering data is probably more reliable than either estimate and corresponds to $\tau = 2.07 \times 10^{-4}\text{cm.}^{-1}$ ($t = 25°$ C. and $\lambda = 5461$ Å).

The measurements were extended to aqueous solutions of crystalline bovine serum albumin (from the Armour Laboratories). In this case the solvent contained a considerable number of small motes, but it was possible to obtain small amounts of clean 1% solutions of the protein by centrifuging in a cold room at 5° C. The increment in turbidity obtained on addition of the solution to the solvent must then be due only to added solute and the results should be reliable. For $\lambda = 5461$ Å, $t = 25°$ C., and using the τ previously determined for benzene as a standard, a value of $\left(\frac{c}{\tau}\right)_{c=0} = 3.73$ was obtained. Insertion of this in Equation (1) along with $\frac{dn}{dc} = 0.187$ (22) yielded a value for the molecular weight = 70,000 (accepted value 69,000). The dissymmetry and depolarization corrections were found to be negligible. This good agreement supports the reliability of the calculated τ of benzene and the osmotic pressure measurements on the polystyrene.

In one experiment the albumin solution was filtered through a fine Pyrex filter. Measurements with this solution indicated a molecular weight increasing from 78,000 to 88,000 during the course of an afternoon. Continued increase of turbidity and dissymmetry was due to the growth of microorganisms indicating another source of error with aqueous solutions.

The Measurement of $\frac{dn}{dc}$

For most high polymers $\frac{dn}{dc}$ is of the order 0.1 to 0.2. The usual practice is to measure the refractive index difference between a 1% solution and the solvent (0.001 to 0.002) and make the reasonable assumption that the difference varies directly with concentration. Since the $\frac{dn}{dc}$ term is squared in Equation (1), an error of one in the fifth decimal for $(\Delta n)_{1\%} = 0.001$ means a 2% error in molecular weight. The extreme limit of accuracy of the Pulfrich Refractometer is 1 to 2×10^{-5}. To obtain this accuracy several modifications and precautions are necessary.

First of all, the same sample of solvent must be used in making up the solution and in the measurements. Appreciable differences in refractive index due to traces of impurities such as water were found between samples of reagent benzene from different bottles. Another precaution is to ensure that solvent

and solution are at the same constant temperature. It is desirable to construct a jacket to fit over the cell of the refractometer and circulate the thermostat water through it as well as the cell holder. The greatest difficulty with the differential cell is that the liquids creep, by capillarity in the corners, from one side to the other. It was found necessary to seal one side from the other with a specially designed polyvinyl alcohol lid in which there were holes with caps for filling. It is necessary to take the average of many readings for each of several fillings of the cell.

It was planned to use the light-scattering apparatus to investigate polymeric aluminum soaps which had been previously studied by means of osmotic pressure and viscosity measurements by Sheffer (26). For these compounds in benzene $(\Delta n)_{1\%}$ is of the order of 0.0004, so that the accuracy of the Pulfrich is obviously insufficient. Greater accuracy can be obtained with the use of interferometry or of specially designed differential refractometers. The differential refractometer designed for this work was similar in principle to that of Debye (9) and Bryce and Speiser (3). The principle is magnification by an optical lever of the slight shift in a beam of light due to refraction at the interface between solvent and solution.

The main feature of the instrument shown schematically in Fig. 5 is that the parallel monochromatic beam of light is refracted four times by making

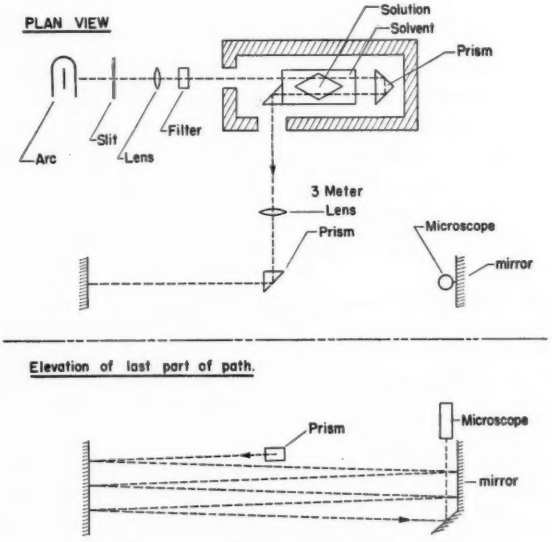

FIG. 5. *Differential refractometer.*

use of a diamond shaped cell and reflecting prisms. This cell is made of stainless steel with glass windows cemented over the holes; the refracting angle is 130°. The cell is filled with 6 cc. of solution and cemented into a

rectangular glass cell containing solvent. This cell is placed in a holder in a box through the walls of which water is circulated from a thermostat. It is necessary to use a good adjustable slit as the source. An image of this slit is formed at the extremity of a 3 m. optical lever and its deflection, from the zero with no cell present, is measured by means of a traveling microscope micrometer. The space required is reduced by reflecting the beam back and forth between two parallel front surface mirrors.

For calibration of this apparatus a 4% solution of cyclohexane in benzene was prepared and the desired refractive index increment obtained on the Pulfrich refractometer. The deflections for several dilutions of this solution were measured on the differential refractometer. Fig. 6 shows the sensitivity

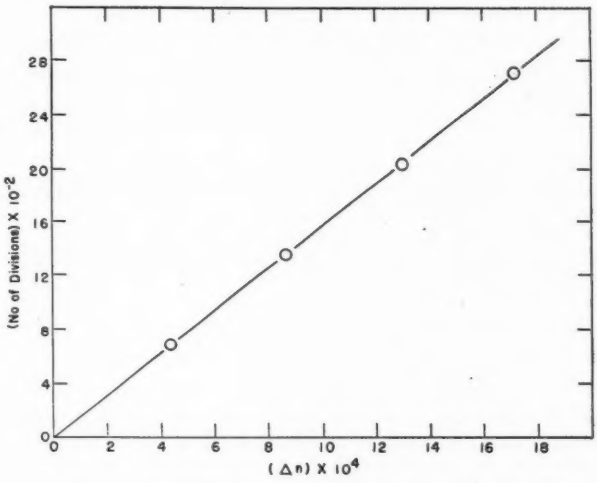

FIG. 6. *Calibration and linearity of differential refractometer.*

and linearity of the apparatus. One division on the micrometer corresponds to a refractive index difference of about 6×10^{-7}. It was necessary to correct the zero for a small shift produced when both cells were filled with solvent. This was because the faces of the rectangular cell were not exactly parallel to one another or perpendicular to the beam. Because of the method used in mounting the components in this apparatus it was found that room temperature variations also caused a small shift in the zero, so that it had to be redetermined after each reading with the cell in position. Two hours was required for the attainment of true temperature equilibrium and readings were continued through the course of the day and finally averaged.

Discussion

The accuracy of the molecular weights obtained by the light-scattering method is limited by the accuracy of the turbidity standard as well as the

experimental errors involved in the measurements. In cases in which it is possible to get solvent and solutions relatively "clean" the latter error should be less than $\pm 5\%$, but, as a rule, for water and aqueous solutions this error is increased, particularly when dissymmetry measurements are required. The error in the directly measured turbidity of benzene is said to be about $\pm 6\%$ (6, 23), while errors in using other methods for calibration of the light-scattering apparatus are considerably greater. It would appear, therefore, that until more accurate measurements of the turbidity of benzene or other standards are made, a possible error of about $\pm 10\%$ must be admitted for molecular weights obtained by light-scattering methods which are comparative in nature.

Acknowledgments

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THE EFFECT ON REACTION RATES CAUSED BY THE SUBSTITUTION OF C^{14} FOR C^{12}[1]

I. THE ALKALINE HYDROLYSIS OF CARBOXYL-LABELED ETHYL BENZOATE

By William H. Stevens and Richard W. Attree[2]

Abstract

A study of the alkaline hydrolysis of C^{14} carboxyl-labeled ethyl benzoate has shown that the substitution of C^{14} for C^{12} changes the rate of hydrolysis of the ester. Ester molecules containing C^{14} hydrolyze at a slower rate than normal ester molecules. The ratio of the hydrolysis rate constants at room temperature has been found to be 0.86 ± 0.016.

Introduction

In the past it has been assumed, in studies using tracer isotopes, that the labeling atoms are chemically indistinguishable from the more abundant normal atoms, except in the case of the hydrogen isotopes, and perhaps lithium. The small differences in the physical properties of compounds containing different isotopes of the same element which make it possible to concentrate isotopic species such as C^{13} and N^{15} in the form of $C^{13}O_2$, $C^{13}H_4$, and $N^{15}H_3$, for example, were considered to have so little effect on normal reactions as to be negligible for ordinary tracer purposes. For instance, the equilibrium enrichment factor for the reaction $HC^{12}O_3^- + C^{13}O_{2(g)} \rightleftharpoons C^{12}O_{2(g)} + HC^{13}O_3^-$ at room temperature, is only 1.012.

Recently, however, it has been found in several laboratories that quite appreciable isotopic effects occur with both C^{13} and C^{14}. Beeck and coworkers (1) were the first to note an effect, and found that the dissociation probabilities of the $C^{12}-C^{12}$ and $C^{12}-C^{13}$ bonds of the molecule-ions formed from propane $-1-C^{13}$ by electron impact differed by about 20%. Later (7) they subjected propane $-1-C^{13}$ to pyrolysis at 500° C. and found an 8% more frequent rupture of $C^{12}-C^{12}$ bonds than of $C^{12}-C^{13}$. Yankwich and Calvin (8) thermally decarboxylated singly carboxyl-labeled malonic and bromomalonic acids, and found that the $C^{12}-C^{12}$ bond ruptured 12% more frequently than the $C^{12}-C^{14}$ bond in malonic, and 41% more frequently than the $C^{12}-C^{14}$ bond in the bromomalonic acid. It should be noted, however, that from a theoretical consideration of the isotopic effect to be expected in these cases, Bigeleisen (2) obtains values that are in disagreement with the experimental values and predicts a considerably smaller effect. Still more recently, Daniels and Myerson (4) hydrolyzed C^{14} labeled urea using the enzyme urease, and found that the carbon dioxide produced early in the hydrolysis had a noticeably higher specific activity than that produced late in the hydrolysis.

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A simple alkaline ester hydrolysis was chosen for the initial study of the effect of C^{14} substitution on reaction rates in this laboratory. The ester used was ethyl benzoate.

Discussion

If the rate of hydrolysis of carboxyl – C^{14} ester molecules were slower, for example, than that of normal C^{12} molecules, the first portion of acid produced in the hydrolysis of labeled ester would contain a smaller percentage of labeled molecules than were present in the ester before hydrolysis began. As hydrolysis continued, acid produced during any short period would contain an increasingly greater percentage of labeled molecules as the normal ester molecules were impoverished more rapidly than the labeled molecules. Very near the end of the hydrolysis the last portion of acid produced would have the greatest concentration of label. Over-all, the complete hydrolysis would show no isotopic fractionation, but total acid produced up to any time before complete hydrolysis would show a specific activity less than that of the acid as a whole.

It was considered that isotopic fractionation might occur only to a slight extent in hydrolysis. Preliminary experiments were therefore done, based on the foregoing reasoning, with a view to observing as large a difference in specific activity as possible. Consecutive samples of benzoic acid as it was produced in an hydrolysis of labeled ester were obtained, and their specific activities determined and compared. The trend in specific activity was noticeably upward, and the specific activity of the last sample of acid was some 20% higher than that of the first, showing that the labeled molecules did hydrolyze at a slower rate than the normal ester molecules. When this magnitude of difference was observed, it was decided to determine the effect more accurately in terms of relative reaction rates.

If an hydrolysis of an ester, a bimolecular reaction, is done with an excess of hydroxyl ion present, kinetically the reaction is first order.

Let

$$k = \frac{1}{t} \ln \frac{C_0}{C_0 - x} \tag{1}$$

and

$$k^* = \frac{1}{t} \ln \frac{C_0^*}{C_0^* - x^*} \tag{2}$$

be the integrated rate expressions for the hydrolysis of the normal and labeled molecules, respectively, where C_0 and C_0^* are the initial concentrations of ester and x and x^* are the concentrations of acid produced up to time t.

Let

$$C_0^*/C_0 = a,\ x^*/x = a', \quad \text{and} \quad a'/a = r\ ,$$

then

$$C_0^* = aC_0 \quad \text{and} \quad x^* = a'x = arx\ .$$

Dividing (2) by (1),

$$\frac{k^*}{k} = \frac{1/t \ln \dfrac{C_0^*}{C_0^* - x^*}}{1/t \ln \dfrac{C_0}{C_0 - x}} .$$

If the hydrolysis is allowed to proceed until $\frac{x}{C_0} = f$

then

$$\frac{k^*}{k} = \frac{\ln \dfrac{C_0}{C_0 - rfC_0}}{\ln \dfrac{C_0}{C_0 - fC_0}} = \frac{\ln \dfrac{1}{1 - rf}}{\ln \dfrac{1}{1 - f}} = \frac{\ln (1 - rf)}{\ln (1 - f)} \qquad (3)$$

When $x^* \ll x$ and $C_0^* \ll C_0$, $\frac{x + x^*}{C_0 + C_0^*} \approx \frac{x}{C_0} = f$.

Further,

$$\frac{x^*}{x + x^*} \approx \frac{x^*}{x} = a', \text{ and } \frac{C_0^*}{C_0 + C_0^*} \approx \frac{C_0^*}{C_0} = a .$$

Therefore

$$r \approx \frac{\dfrac{x^*}{x + x^*}}{\dfrac{C_0^*}{C_0 + C_0^*}} ,$$

which is the ratio of the specific activity of the reacted fraction to that of the initial ester.

Thus the ratio of the reaction rates, for a first order reaction, may be determined readily if the initial specific activity, the specific activity of a reacted fraction, and the fraction reacted are known, when material having low specific activity is used.

Experimental

Benzoic acid, carboxyl – C^{14}

Various techniques for preparing carboxyl-labeled acids by carbonation of the appropriate Grignard reagent with labeled carbon dioxide have appeared in the literature. A summary of most of these appears in "Isotopic Carbon" (3). Carboxyl-labeled benzoic acid has been prepared by Dauben, Reid, and Yankwich (5).

Phenylmagnesium bromide (10 millimoles) was prepared in a 50 ml. round bottomed flask under nitrogen, using a Rosinger magnetic stirrer.* The Grignard was frozen by immersing the flask in liquid nitrogen. The flask was then connected to a high vacuum line and evacuated to 10^{-3} mm. Carbon dioxide was generated in the vacuum system by heating labeled barium

* *Essentially a short rod of Alnico steel driven by a rotating magnet. We have found this to be much simpler than the complicated induction stirrer used in high vacuum systems by many workers.*

carbonate with eight to ten times its weight of lead chloride to about 350° C. (6). The carbon dioxide was frozen down into the Grignard flask, using liquid nitrogen. The flask was isolated from the vacuum line by closing a stopcock; the mixture was allowed to melt and was vigorously stirred. The freezing, melting, and stirring cycle was repeated. The addition product was then hydrolyzed with dilute sulphuric acid, and the benzoic acid extracted with ether. The acid was extracted from the ether into 1 *N* sodium hydroxide, precipitated with 6 *N* hydrochloric acid, and filtered on sintered glass. Yields were 80 to 85%, based on barium carbonate. Radiochemical yields were not determined.

Ethyl benzoate, carboxyl – C^{14}

The ester was prepared through the acid chloride. Benzoic acid (10 millimoles) was heated with an excess of thionyl chloride (25 millimoles). When evolution of hydrogen chloride ceased, the excess thionyl chloride was distilled off under slightly reduced pressure, through a small fractionating column. An excess of absolute alcohol (40 millimoles) was added and the mixture refluxed for two hours. The excess alcohol was distilled at atmospheric pressure, then the ester fractionated at reduced pressure.

Alkaline hydrolysis of ethyl benzoate, carboxyl – C^{14}

Ethyl benzoate (9 millimoles) was hydrolyzed in 100 ml. of a solution 50% by volume alcohol and 0.5 *N* with sodium hydroxide, for a short period. One-half of the solution was then diluted with 200 ml. of water and extracted with ether to remove unhydrolyzed ester. The aqueous solution was acidified and the benzoic acid extracted with ether. The ether layer was washed with water and evaporated under slightly reduced pressure in a stream of nitrogen. The benzoic acid was taken up in 15 ml. of alcohol and divided into two fractions of 5 and 10 ml. respectively. These fractions were titrated with 0.05 *N* sodium hydroxide, using phenol red indicator. Each fraction was then diluted to 25 ml. with water containing 1% of hydrazine hydrate. After hydrolysis was completed in the second half of the main solution, a 6 ml. sample was withdrawn. The benzoic acid was isolated and divided into two fractions in the same way as before. After titration, they were diluted to 25 ml. also. These four sodium benzoate solutions were used for counting.

Samples for volumetric analysis for benzoic acid were taken from the main solution at the end of the short hydrolysis period, and after complete hydrolysis, to determine the fraction of ester hydrolyzed in the initial hydrolysis period.

The radiochemical purity of the benzoate solutions was checked in the following manner. After samples of the benzoate solutions had been counted, each solution was acidified with sulphuric acid and the benzoic acid re-extracted with ether. The ether solutions were washed with water twice and the benzoic acid was isolated and sodium benzoate solutions prepared for counting as before. The specific activities remained constant.

Sample Mounting for Counting

The benzoic acid was counted as sodium benzoate. Samples of 50λ or 100λ of the sodium benzoate solutions obtained were pipetted onto stainless steel disks. The disks were 2.5 cm. in diameter, having an area of 3.45 cm.^{2} defined by a thin, 2 mm. wide, ring of Tygon paint primer around the outside rim. The samples were dried under an infrared lamp, with a gentle stream of air directed toward the disk. With hydrazine hydrate present, the solutions spread fairly well, and, with a little care, a uniformly thin layer of salt was obtained. Sample thickness ranged from 0.05 to 0.2 mgm. per cm.^{2}, the actual thickness depending on the fraction and sample volume.

Counting

The samples were counted in a Simpson type, methane filled, proportional counter with linear amplifier (National Research Council, Atomic Energy Project, "Methane, low level β-counter assembly, 505A"). In this type of instrument the count recorded is relatively insensitive to changes in area of samples being counted, as long as the thickness of any sample is very small and is reasonably uniform; in other words, as long as self-absorption is negligible. The geometry obtained is slightly greater than 50% because of back-scattering. At the sample thickness used, self-absorption was negligible, since a constant counting rate per milligram of material was obtained when the thickness was varied by a factor of four.

Results

Analytical and counting results are summarized in Table I.

TABLE I

EXPERIMENTAL RESULTS

Material sampled	Time of sampling	Weight of acid, gm.	Specific activity, counts/min./mgm.
Acid produced in initial hydrolysis	5 min.	0.112	$a' = 8{,}179 \pm 80$
Acid produced by complete hydrolysis	72 hr.	1.11	$a = 9{,}427 \pm 115$

From these experimental values, $f = 0.101$ and $r = 0.87 \pm 0.016$. Using Equation (3), $k^*/k = 0.86 \pm 0.016$ or $k/k^* = 1.16 \pm 0.017$, where the limits shown represent the errors in internal consistency in sampling and counting.

Conclusion

From these results, and those of others noted in the introduction, it becomes obvious that in the case of C^{14} care must be taken in the quantitative interpretation of data obtained in tracer experiments wherever all reactions do not go essentially to completion.

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